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ORIGINAL PAPER

Nursery practice on seed germination and seedling growth of *Dalbergia sissoo* using beneficial microbial inoculants

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Abstract : Nursery practice using microbial inoculants was performed to find out the efficacy of the inoculants on seed germination and seedling growth of sissoo (*Dalbergia sissoo* Roxb.). Microbial inoculants or effective microorganisms (EM) are a mixture of many different beneficial microorganisms in a solution. The seedlings were grown in a mixture of sandy soil and cowdung (3:1) kept in polybags with pouring EM solution at different concentrations (0.1%, 0.5%, 1%, 2%, 5% and 10%) before and after a week of sowing the seeds. Seed germination rate and growth parameters of seedlings were measured, such as, shoot and root length, vigor index, fresh and dry weight of shoot and root and total biomass increment. The nodulation status influenced by EM was also observed along with the measurement of pigment contents in leaves. The highest germination rate (69%) was observed in 2% EM treatment, followed by 67% and 65% in 1% and 5% EM. The highest shoot length (33.2 cm) was in 2% EM, whereas highest root length (26.3 cm) was in 1% EM. Both fresh and dry weights from shoot and root, were maximum (4.16 g and 1.57 g; 2.12 g and 0.83 g respectively) in 2% EM and were significantly ($p \leq 0.05$) different from control. Vigor index was highest (4071) in 2% EM, which was significantly ($p \leq 0.05$) different from control. Total dry weight increment was highest in 2% EM treatment, followed by 1% and 5% concentrations of EM. Nodulation number was higher at very low (0.1%) concentration of EM solution but it normally decreased with the increase of EM concentrations. The contents of chlorophyll a, chlorophyll b and carotenoid were highest (60.11, 17.05 and 42.48 mg·L^{-1}respectively) in 2% EM treatment and lowest (39.35, 13.55 and 27.29 mg·L^{-1} respectively) in control treatment. Therefore, low concentration of EM (up to 2%) can be used for getting maximum seed germination rate and seedling development of *Dalbergia sissoo* Roxb.

Keywords: *Dalbergia sissoo* Roxb.; microbial inoculants (EM); germination rate; seedling growth; leaf's pigment; nodulation status

Introduction

Sissoo (*Dalbergia sissoo* Roxb.) is an important multipurpose tree species under the family Leguminosae (Papilionaceae). It is also a medium sized to large, fast growing and gregarious deciduous tree with a spreading crown. The tree is considered to be native of Bengal and found in India, Nepal, Bhutan, Bangladesh, Myanmar, Malaysia, Pakistan and Afghanistan. It is also found under cultivation in tropical to subtropical Africa and Asia. Sissoo has been introduced into Java, Nigeria, Mauritius, Sri Lanka, Kenya, Northern Rhodesia, Palestine and Union of South Africa with varying degree of success (Tewari 1994). It is being planted in different parts of Bangladesh by the government and other public and private sectors in different plantation programs like, Agro-forestry, Community Forestry, Social Forestry, Village and Farm Forestry Programme, etc. To fulfill the high demand of large number of seedlings, many organizations are producing sissoo seedlings in the nursery in Bangladesh. Artificially, as the plants are grown in unfavorable soil conditions, beneficial soil microorganisms like EM (microbial inoculants) can play a significant role in early establishment and better growth of the inoculated seedlings under field conditions.

The microbial inoculant in this study, with a commercial name as "Effective Microorganisms" or EM, was developed at the University of Ryukyus, Okinawa, Japan, in the early 1980's by a distinguished Professor of Horticulture, Dr. Teruo Higa (Kyan et al. 1999). The main microbial species comprising EM are lactic acid bacteria, photosynthetic bacteria, beneficial fungi, yeast, ray fungi and others. The microorganisms are combined into the inoculant in the manufacturing process and can survive in the inoculant liquid at pH 3.5 or below. The density of most of the above mentioned microbes is in the range of 1×10^6 to

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1×10^8 individual·mL^{-1} (Xu 2000), which can be applied as inoculant to increase the microbial diversity of soils. It has been used with considerable success to improve soil quality and yield of crops particularly in nature farming and organic farming systems (Xu 2000). Hence, the inoculation of EM cultures to the plants can improve their photosynthesis and fruit yield (Xu 2000; Wang et al. 2000). Although *D. sissoo* is used for wide range of purposes and even planted intensively in the field, the initial growth potential under the influences of microbial inoculants was not studied. Therefore, the objective of this study was to observe the effectiveness of EM inoculant on seed germination and the seedling growth of sissoo and also to find out the best concentration of EM solution for ensuring maximum seedling development in the nursery.

Materials and methods

The experiment was carried out in the nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong, Bangladesh (Lies approximately at the intersection of 91°50′E and 22°30′N). The seeds of *D. sissoo* were collected from the Seed Orchard Division of Bangladesh Forest Research Institute (BFRI). The soils collected from the degraded hills of the University Campus was sieved well (<3 mm) and mixed with decomposed cowdung in a ratio of 3:1. The brown hill soils (Rashid 1991) are sandy loam to sandy clay loam, moderately to strongly acid and poorly fertile (Osman et al. 2001). The polybags of 15 cm × 10 cm in size were filled with the prepared mixture. A thin layer of coconut husk was provided in each of the polybags as a top layer of the polybag media to reduce the evaporation and to supply a source of organic matter. There were seven treatments including control and 20 replications for each treatment. Seeds sown at polybag were not added with EM but water only for control and seeds sown at polybag soil were added with 0.1%, 0.5%, 1%, 2%, 5% and 10% concentrations of EM solution. The 50-mL solution of EM was mixed with the soils before one week of sowing the seeds and another 50 mL was applied after one week of sowing the seed in separate polybags. Three seeds were sown in an individual polybag to observe the influence of EM on germination. After completing germination, only one seedling per polybag was maintained for observing the growth parameter and nodulation status of seedlings. Partial shade and covering were provided using polythene on nursery roof to protect the seedlings from strong sunlight and rain.

Germination rate was recorded daily from the date of seed sowing and continued up to the last germination (12 d). Then the seedlings were allowed to grow altogether for 3 months from the time of the last germination of seeds. After 3 months, five representative seedlings from each treatment were selected for measuring their growth parameters. The recorded parameters included shoot and root length, collar diameter, leaf number, fresh shoot and root weight, dry shoot and root weight and nodulation status. For recording dry weight, shoots and roots were oven dried at 70°C until the constant weight was obtained. Vigor index was also calculated in this experiment.

The pigment contents (chlorophyll a, chlorophyll b and carotenoid) were determined from the fresh leaves of seedlings in different treatments. Ten leaf discs were cut from leaves with a cork borer (inside diameter of 5 mm), weighted immediately after cutting and dipped in 100% acetone of 5 mL in test tube with stopper. After 24 h of incubation, the supernatant colored solution from the top was decanted carefully in 25-mL volumetric flask. The leaf discs were then crushed with a blunt glass rod gently and 5-mL fresh acetone was added to the test tube and left for 15 min. Then the supernatant colored solution from the top was again decanted to the same volumetric flask very carefully, avoiding the fragmented plant tissues. The process was repeated until the leaf fragments became colorless. Finally the volume was made up to 25 mL with fresh acetone and measurement was taken immediately after the preparation of solution. The measurement of chlorophyll a, chlorophyll b and carotenoid was made at 662 nm, 644 nm and 440.5 nm respectively, with a spectrophotometer (Spectronic-20). The pigment contents in the extract were calculated by following the formula of Wettstein (1957).

$$C_a = \frac{(9.784E662 - 0.99E644)\times V \times d}{1000F_w} \quad (1)$$

$$C_b = \frac{(21.426E644 - 4.650E662)\times V \times d}{1000F_w} \quad (2)$$

$$C_c = \frac{[4.695E440.5 - 0.268(C_a + C_b)]\times V \times d}{1000F_w} \quad (3)$$

where, C_a is the chlorophyll a (mg·L^{-1}), C_b the chlorophyll b (mg·L^{-1}), C_C the carotenoid (mg·L^{-1}), V the total volume (25 mL), L the litter of leaf extract solution, d the dilution factor, F_W the fresh weight of leaf disc (g), and E is the absorbance at a particular wavelength (440.5, 644 and 662 nm).

All data were analyzed statistically using the computer software package SPSS (Version 13, SPSS Incorporation, Chicago, USA). Possible significant variations among the treatments were explored by Duncan's Multiple Range Test (DMRT).

Results and discussion

The highest germination rate (69%) was observed in 2% EM treatment, followed by 67% and 65% in 1% and 5% EM treatments. The shoot length (33.2 cm) was highest in 2% EM, whereas highest root length (26.3 cm) was found in 1% concentration EM solution. Collar diameter was highest (5 mm) in 5% EM treatment and was significantly ($p \le 0.05$) different from that of control. Seedlings treated with EM had more leaves, compared with control (Table 1).

Both fresh and dry weights from shoot and root, were maximum (4.16 g and 1.57 g; 2.12 g and 0.83 g respectively) in 2% EM treatment and were significantly ($p \le 0.05$) different only from that in control treatments. In all the cases, lowest growths

(Fresh and dry weights of both shoot and root) were observed in control treatments (Table 2). Vigor index was highest (4071) in 2% EM, followed by 1% and was significantly ($p \leq 0.05$) different from that of control treatment (Table 1). Total dry weight increment was highest in 2% EM treatment, followed by 1% and 5% EM. Increased biomass production (shoot and root weights) in the treated seedlings might be due to the better root development. Such promotion might also be due to biological active substances in EM solution (Lim et al. 1999) such as IAA and gibberellins, which positively improves plant growth (Chowdhury et al. 1994).

Table 1. Effect of different concentrations of effective microorganisms (EM) on seed germination rate, shoot and root length, vigor index, collar diameter and leaf number of sissoo (*Dalbergia sissoo*) after three months from the time of the last germination of seeds in the nursery

Concentration of EM (%)	Germination rate (%)	Length (cm)			Vigor index	Collar dia. (mm)	Number of compound leaf
		Shoot	Root	Total			
Control	52 b *	26.7 b	21.1 b	47.8 b	2486 c	3.9 b	15.2 b
0.1	59 ab	28.1 ab	23.9 ab	52.0 b	3068 b	4.2 b	16.0 a
0.5	63 ab	29.3 ab	24.1 a	53.4 b	3364 ab	4.5 a	16.6 a
1	67 a	32.5 a	26.3 a	58.8 a	3940 a	4.7 a	17.4 a
2	69 a	33.2 a	25.8 a	59.0 a	4071 a	5.0 a	16.8 a
5	65 a	31.8 a	24.5 a	56.3 a	3660 ab	4.8 a	16.4 a
10	61 ab	29.6 ab	23.7 ab	53.3 b	3251 b	4.4 a	16.2 a

Notes: *_Means followed by the same letter (s) are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).

Table 2. Effect of different concentrations of effective microorganisms (EM) on fresh and dry weight of shoot and root of sissoo (*D. sissoo*)

Concentration of EM (%)	Fresh weight (g)			Dry weight (g)			Total increment (%)
	Shoot	Root	Total	Shoot	Root	Total	
Control	3.03 b *	0.99 b	4.02 b	1.55 b	0.52 b	2.07 b	00.00
0.1	3.27 ab	1.22 ab	4.49 ab	1.64 ab	0.63 ab	2.27 ab	+9.66
0.5	3.42 ab	1.35 ab	4.77 ab	1.73 ab	0.72 ab	2.45 ab	+18.36
1	3.79 a	1.54 a	5.33 a	1.96 a	0.81 a	2.77 a	+33.82
2	4.16 a	1.57 a	5.73 a	2.12 a	0.83 a	2.95 a	+42.51
5	3.84 a	1.46 a	5.30 a	1.91 a	0.75 a	2.66 a	+28.50
10	3.56 ab	1.33 ab	4.89 ab	1.87 ab	0.71 ab	2.58 ab	+24.64

Notes: *_Means followed by the same letter (s) in the same column do not vary significantly at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).

Effective microbial inoculants are being applied in Japan, USA, France, China, Brazil, Thailand and many other countries of the world. Application of EM can play an important role in enhancing seed germination, seedling growth and yield of various agricultural crops and vegetables (Iwaishi 2000; Shin et al. 1995; Vongprachanch 1995; Zacharia 1995). EM solution with organic fertilizer and other chemicals is also reported to enhance seed germination, seedling growth and yield of different agricultural crops (Ahmed et al. 1995; Anuar et al. 1995; Xu 2000). But the influence of EM on the forest crop has not been studied widely (Khan et al. 2005, 2006). From this study on forest crop, it has also been observed that soils amended with different concentrations of EM can improve the seedling growth. Khan et al. (2006) also reported that at lower concentration of EM, seed germination rate was enhanced by EM treatment. However, as the concentration of EM increased, seed germination rate was depressed, which may be due to toxicity of higher concentrations of EM.

Nodule number was highest (39) in 0.1% EM treatment, followed by 37 in 0.5%, 33 in control and 32 in 1% EM. Nodule number was lowest (24) in 10% EM. Both fresh and dry weights of nodule were also maximum (0.46 g and 0.15 g respectively) at 0.1% EM and lowest in 10% EM. Number and dry weight of nodule were increased in 0.1% and 0.5% EM, compared with other treatments (Table 3). The increment rate of fresh weight was positive in case of 0, 0.5% and 1% EM and negative in 2%, 5% and 10% EM, compared to control (Table 3). These results support the finding of Thach et al. (1999) that number of nodule in soybean roots was not significantly increased due to application of higher concentrations of EM.

Table 3. Effect of different concentrations of effective microorganisms (EM) on nodule number and their fresh and dry weight of sissoo (*D. sissoo*)

Concentration of EM (%)	Nodule					
	Number	Weight (g)		Ratio of increased to decreased	Weight ratio of increased to decreased	
		Fresh	Dry		Fresh	Dry
Control	33 a*	0.37 ab	0.13 a	00.00	00.00	00.00
0.1	39 a	0.46 a	0.15 a	+18.18	+24.32	+15.38
0.5	37 a	0.44 a	0.14 a	+12.12	+18.92	+7.69
1	32 a	0.38 a	0.12 ab	-3.03	+2.70	-7.69
2	28 b	0.36 ab	0.11 ab	-15.15	-2.70	-15.38
5	27 b	0.32 ab	0.10 ab	-18.18	-13.51	-23.08
10	24 b	0.29 b	0.9 b	-27.17	-21.62	-30.77

Notes: *_Means followed by the same letter (s) in the same column do not vary significantly at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).

Effects of EM on the contents of leaf's pigments (Chlorophyll a, chlorophyll b and carotenoid) were also determined. The con-

tents of chlorophyll a, chlorophyll b and carotenoid were highest (60.11, 17.05 and 42.48 mg·L^{-1} respectively) in 2% EM and lowest (39.35, 13.55 and 27.29 mg·L^{-1} respectively) in control treatment (Table 4). Total pigment content was highest (119.64 mg·L^{-1}) in 2% EM and was significantly ($p \leq 0.05$) different from that in other treatments, including control. The present results are in agreement with other findings (Xu 2000, Wang et al. 2000, Mridha et al. 2002). Low concentration of EM with organic fertilizers promotes root growth and activity further to enhance photosynthetic efficiency and yield of seedlings. Hence, from the present information it can be concluded that the low concentration of EM solution (up to 2%) can be used for getting maximum seed germination rate and seedling development of sissoo in the nursery, which may also be effective for seedling growth in the field.

Table 4. Effect of different concentrations of effective microorganisms (EM) on pigment contents in fresh leaves of sissoo (*D. sissoo*)

Concentration of EM (%)	Contents of pigments (mg·L^{-1})				Total pigment increment (%)
	Chloro-phyll a	Chlorophyll b	Carete-noids	Total	
Control	39.35 c *	13.55 b	27.29 c	80.19 d	00.00
0.1	43.46 c	14.43 ab	35.76 b	93.65 c	+16.83
0.5	45.38 c	15.40 ab	36.24 b	97.02 bc	+21.04
1	52.93 b	16.12 a	39.39 a	108.44 ab	+35.31
2	60.11 a	17.05 a	42.48 a	119.64 a	+49.31
5	58.52 a	14.33 ab	40.13 a	112.98 a	+40.99
10	49.70 b	15.81 a	38.12 a	103.63 ab	+29.30

Notes: *_Means followed by the same letter (s) in the same column do not vary significantly at $p \leq 0.05$, according to Duncan's Multiple Range Test (DMRT).

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